SYNOPSIS OF RESEARCH PROJECT

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6. Minor Subject	**:**	Molecular Biology and Biotechnology
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8. Title of Research Project	**:**	**ASSOCIATION MAPPING FOR YIELD-RELATED TRAITS AND IDENTIFICATION OF LINKED MARKERS IN CHICKPEA (*Cicer arietinum L.*)**

9. Introduction

The chickpea (*Cicer arietinum L.*), a member of the Leguminosae family and one of humanity's oldest pulse crops, is a diploid species with a compact genome of approximately 738 Mb (Varshney *et al.*, 2013) that ranks third in global legume production behind dry beans and field peas. While it originated in south-eastern Turkey and Syria, it is now cultivated in over 50 countries and categorized into the smaller, darker Desi type and the larger, cream-colored Kabuli type. Often dubbed "the meat of the poor," this nutritional powerhouse provides 20%–25% high-quality plant protein, essential vitamins, and critical minerals like iron and phosphorus, making it vital for managing cardiovascular health and Type 2 diabetes. Beyond nutrition, the chickpea is a cornerstone of sustainable dryland agriculture; through symbiosis with *Mesorhizobium* bacteria, it performs biological nitrogen fixation (BNF) to add up to 140 kg N/ha to the soil, reducing reliance on synthetic fertilizers while utilizing its deep taproot system to flourish in moisture-stressed environments.

Chickpea remains a cornerstone of global trade and a vital source of plant-based protein. In 2025–2026 marketing year, global production is forecasted to reach approximately 17.8 million tonnes, a 1.4% increase over the previous season (Anonymous, 2025). India continues to dominate the sector, with the Ministry of Agriculture's third advance estimates projecting a production of 11.34 million metric tonnes (MMT) for 2025–26 (Anonymous, 2025a). This growth is supported by an increase in the Rabi sowing area, which reached 95.88 lakh hectares by early 2026, 5.11% rise compared to the previous year (Anonymous, 2026). In spite of these advancements, diminished crop productivity remains a formidable obstacle, as significant yield losses continue to hinder the crop's full economic potential. While theoretical yields are estimated at 6.0 t/ha, actual global averages fluctuate between 1.0 and 1.2 t/ha. In the current agricultural landscape of 2026, terminal drought and heat stress continue to threaten productivity, with potential yield losses still exceeding 50% in moisture-stressed environments (Anonymous, 2025b). To mitigate these risks, modern breeding programs are increasingly focusing on genomic tools and high-yielding varieties such as D-3113 and D-1113, which show high heritability for seed yield under semi-arid conditions (Anonymous, 2026a).

To address these challenges, breeding programs must move beyond traditional methods by integrating robust statistical and genomic tools. The foundation of this selection process rests on the concept of heritability, formally defined by Jay L. Lush in 1940 as the proportion of phenotypic variance attributable to additive genetic effects; however, as established by Johnson, Robinson, and Comstock (1955), high heritability alone is an insufficient predictor of breeding progress and must be coupled with Genetic Advance (GA) to accurately estimate "genetic gain"—the actual improvement achievable through selection over successive generations. Understanding the relationships between grain yield and its contributing traits requires a sophisticated analytical approach because, as Sewall Wright noted in 1921, simple correlation can be deceptive as it fails to distinguish between cause and effect. By employing Wright's path coefficient analysis (often referred to as route analysis) to "unpack" correlation coefficients into direct and indirect effects, researchers can discern whether a trait, such as "primary branching," contributes directly to yield or is merely an indirect consequence of an increase in the "total number of pods," thereby combining Pearson's correlation with path analysis to provide a biological roadmap for prioritizing morphological traits that ensure consistent productivity gains.

Complementing these statistical models is the strategic use of molecular markers. While high-density Single Nucleotide Polymorphic (SNP) arrays are common in modern genomics,

Simple Sequence Repeat (SSR) markers remain indispensable for applied plant breeding. Their continued relevance is due to their affordability, multi-allelic nature, and high levels of polymorphism. In this investigation, SSRs were selected as reliable, codominant markers that can be utilized in standard laboratory settings without the need for expensive, large-scale sequencing infrastructure. Identifying meaningful Marker-Trait Associations (MTAs) through SSR profiling offers "breeder-ready" tools for Marker-Assisted Selection (MAS). This approach facilitates the rapid identification of high-yielding genotypes during early growth stages, significantly accelerating the breeding cycle compared to labor-intensive field evaluations.

10. Practical Utility of the Research Project

We are moving away from the slow, unpredictable nature of traditional field trials toward a more precise approach called Marker-Assisted Selection (MAS). By identifying specific genetic markers through association mapping, we can now screen thousands of plants while they are still seedlings. This approach bypasses the inconsistencies caused by climate change, shortens the breeding cycle by several years, and significantly lowers the high costs of manual labor. A key part of this journey involves "mining" for superior genetic traits that were lost over centuries of domestication. Because modern chickpeas have a very narrow genetic foundation, we are exploring a diverse range of genotypes to rediscover overlooked variations in plant structure and yield. This allows us to create a genomic roadmap for developing tougher, high-yielding varieties that can withstand terminal drought and extreme heat.

On the farm, the focus is shifting toward "machine-friendly" chickpeas. As manual labor becomes more expensive and harder to find, developing plants with an upright growth habit and higher pod clearance is an economic necessity. Transitioning to mechanical harvesting can cut costs by 60–80% (Chaudhary *et al.*, 2022), reducing the labor required from 32 man-hours to just 3.5 hours per hectare—a staggering 88% reduction in time (Delvadiya *et al.*, 2024). To make the breeding process more scientific, we use Path Coefficient Analysis to look past the environmental "noise." By pinpointing which specific traits, like seed weight or pod count, most directly impact the final harvest, we can make more predictable selection decisions. Finally, we have strategically chosen SSR markers because they are reliable and affordable. Unlike expensive, high-tech sequencing that requires massive infrastructure, these "breeder-friendly" tools can be used in standard labs anywhere. This ensures that even local breeding programs can access modern technology, helping to stabilize food production and support global food security for everyone.

11. Objectives

Keeping this in view, the present investigation on "Association Mapping for Yield-Related Traits and Identification of Linked Markers in Chickpea (*Cicer arietinum L.*)" will be undertaken with the following objectives:

1. To estimate genetic variability, heritability and genetic advance for yield and yield-attributing traits
2. To study inter-relationship among grain yield and their component traits
3. To assess the nature of G x E interaction and stability of chickpea genotypes over the environment
4. To assess the extent of genetic diversity among genotypes using molecular markers
5. Association mapping for yield and yield attributing traits using molecular markers
6. To study the identification of mechanical harvestable genotypes.

12. Review of Literature

To ensure a thorough understanding of the subject, this section reviews the relevant literature covering the various aspects of the present investigation. The content is organized into the following subheadings to provide a systematic and comprehensive foundation for the study.

12.1 Variability parameters

Manasa *et al.* (2020) focused on the genetic variability within thirty kabuli chickpea genotypes, identifying significant diversity in reproductive traits. Their analysis revealed high levels of both genotypic and phenotypic coefficients of variation (GCV and PCV) for the number of pods per plant and the number of seeds per pod. Furthermore, the study recorded high heritability combined with substantial genetic advance for key parameters such as seed yield, harvest index, plant height, and flowering time. These findings indicate that these traits are primarily governed by additive gene action; therefore, direct selection represents a highly effective strategy for the genetic improvement of these specific kabuli lines.

Deepika *et al.* (2021) utilized an analysis of variance (ANOVA) to examine nine quantitative traits across 71 accessions from the ICRISAT chickpea mini-core collection. Their findings revealed substantial genetic diversity for five key parameters: plant height, pod and

seed counts per plant, and overall pod and seed yield. Across all traits, the phenotypic coefficient of variation (PCV) consistently exceeded the genotypic coefficient of variation (GCV), suggesting that environmental factors played only a minor role in trait manifestation. Notably, pod and seed yields exhibited the highest GCV values. While broad-sense heritability varied—peaking for pod yield and remaining high for plant height and branching—traits like 100-seed weight and flowering time showed lower heritability. Furthermore, high genetic advance as a percentage of the mean was observed for yield-related components and branch number, indicating strong potential for selection-based improvement.

Sriraj and Gurjar (2022) identified varying levels of genetic variability across several traits in a study of twenty genetically distinct chickpea genotypes, which included the control varieties GNG 1488, GNG 158, and PNG 469-20. Moderate levels of both phenotypic and genotypic coefficients of variation (PCV and GCV) were observed for secondary and primary branching, 100-seed weight, harvest index, biological yield, pod count, plant height, and overall seed yield. Conversely, traits such as seeds per pod, protein content, and duration to maturity and 50% flowering exhibited low PCV and GCV values. These lower figures suggest a narrower genetic base for these specific characteristics, potentially limiting the effectiveness of direct selection for crop improvement within the studied population.

Aung *et al.* (2023) highlighted substantial genetic diversity in both desi and kabuli chickpea genotypes, particularly regarding biological yield, pod count, and seed yield per plant. These traits exhibited high phenotypic and genotypic coefficients of variation (PCV and GCV), with elite desi lines showing significant phenotypic superiority, making them ideal candidates for future breeding programs.

Lambani *et al.* (2023) utilized analysis of variance to confirm significant variability across a broad range of traits, from phenological markers—such as flowering and maturity dates—to physical characteristics, including plant height, branching, and seedling vigor. Notably, while PCV values were consistently higher than GCV values for all studied traits (indicating environmental influence), total pods per plant and seed yield per plot demonstrated high heritability coupled with high genetic advance. This specific combination suggests that these traits are primarily governed by additive gene action, making them highly responsive to direct selection in crop improvement efforts.

Mihoariya *et al.* (2023) evaluated 83 genotypes and observed significant genotypic and phenotypic coefficients of variation (GCV and PCV) for biological yield and pod count,

suggesting a rich genetic reservoir within the material. Recent studies have consistently identified robust genetic variability and high potential for selection in chickpea yield components through detailed statistical analysis. Their findings further highlight that traits such as 100-seed weight and seed yield per plant—characterized by high heritability and genetic advance—are prime targets for selecting superior genotypes.

Dileep *et al.* (2024) confirmed that seed yield per plot and biological yield per plant reached peak GCV and PCV values. The study specifically noted that the combination of high heritability and genetic advance for these yield parameters, alongside the number of primary branches, offers a reliable pathway for crop improvement.

Kumar *et al.* (2024) quantified these relationships, reporting the highest variability in pods per plant (GCV 12.92%; PCV 18.47%), followed closely by seed and biological yield. Their data emphasized exceptional heritability for 100-seed weight (96.41) and seed yield (84.11), while pods per plant showed the most substantial genetic advance (26.24). Collectively, these researchers conclude that because these critical yield traits are governed by strong genetic factors, direct selection remains a highly effective strategy for modern chickpea breeding programs.

12.2 Correlation coefficient and Path coefficient analysis

Upadhyay and Lavanya (2019) emphasize that economic and biological yields should be the primary focus for effective selection in chickpea. Their investigation demonstrated that plant height, pod count, seed index, and harvest index are positively correlated with seed yield. Specifically, at the phenotypic level, harvest index (0.713), seed index (0.611), and pods per plant (0.981) showed strong positive associations, while negative correlations were observed for days to 50% pod setting (-0.273), primary branches (-0.312), and days to 50% flowering (-0.312). These trends were even more pronounced at the genotypic level, where the correlation for pods per plant reached 0.994 and harvest index reached 0.793, reinforcing the genetic basis of these relationships.

Yadav *et al.* (2020) utilized correlation and path coefficient analysis to determine yield drivers sixty chickpea varieties. Their results indicated that nearly all components, except for plant height, were positively linked to seed yield. Notably, seed yield showed highly significant correlations with biological yield (0.946) and secondary branches (0.606), as well as positive associations with primary branches (0.302), pods per plant (0.590), and seeds per pod (0.283). Through path coefficicent analysis, the researchers concluded that biological

yield, harvest index, secondary branching, and flowering time are the most critical traits to prioritize for enhancing chickpea productivity.

Jain *et al.* (2021) evaluated the associations among 14 yield-related characteristics across 30 advanced chickpea lines, identifying several critical drivers of productivity. Their analysis revealed a robust and positive correlation between various yield-contributing traits and overall seed production per plant. Conversely, they noted a significant negative correlation between harvest index and seed yield within this specific set of genotypes, a finding that contrasts with some other studies and highlights the unique architectural dynamics of these advanced lines.

Raju and Lal (2021) established that traits such as plant height, primary branching, seeds per pod, 100-seed weight, and total biological output are all favorably linked to grain production, seed yield serves as the most vital economic indicator in chickpea, showing significant positive associations with various growth and yield components at both the phenotypic and genotypic levels. Notably, the study revealed that both seed yield and harvest index exert a substantial direct positive influence on final seed output, underscoring a critical interconnection between a plant's overall biological efficiency and its reproductive capacity. Consequently, the authors conclude that enhancing organic production per plant is an effective strategy for simultaneously driving up economic seed yields.

Lavanya *et al.* (2022) established that seed yield per plant is significantly and positively correlated with several key traits at both the genotypic and phenotypic levels, including plant height, seed index, biological yield, and the number of pods and secondary branches per plant. Specifically, their data highlighted strong positive associations between grain yield and the number of seeds per plant (0.683), harvest index (0.622), and pod count (0.581), with more moderate but significant links to seeds per pod and seed index. In contrast, phenological traits such as days to maturity and pod setting showed only marginal, non-significant positive correlations, while days to 50% flowering exhibited a slight negative association (-0.0415). Ultimately, both correlation and path analysis confirmed that plant height, seed index, and harvest index are the most critical determinants of productivity, suggesting they should be prioritized as primary selection indices for developing improved chickpea varieties.

Pravallika and Lavanya (2022) reported that correlation coefficient analysis revealed significant positive phenotypic and genotypic associations between seed yield per plant and several traits, including harvest index (0.84 and 0.87), biomass (0.52 and 0.50), and the number

of seeds per pod (0.36 and 0.34). Furthermore, path analysis indicated that harvest index, biological yield, shoot fresh weight, number of seeds per pod, number of pods per plant, and seed index all exerted positive direct effects on seed yield.

Ningwal *et al.* (2023) analysed 57 genotypes of chickpea, the researchers found that seed yield per plant is driven primarily by biological yield, the number of pods per plant, and the number of secondary branches. These traits showed strong, positive associations with overall productivity.

Kumar *et al.* (2024) quantified the strength of these relationships, identified biological yield per plant and pods per plant as the most critical determinants of yield. Other contributing factors included the number of seeds per pod and 100-seed weight and marking them all as essential selection criteria for breeders.

Maring *et al.* (2024) observed strong genetic correlations between grain yield and factors such as the harvest index (0.964), seed weight (0.929), and root length (0.878). Notably, their study also indicated that higher yields are often associated with shorter maturation periods and reduced plant height, as evidenced by negative correlations with days to maturity and overall height. Collectively, these studies suggest that selection strategies focusing on high biological yield, pod density, and harvest index while monitoring maturity timing can effectively drive yield improvements.

12.3 Stability analysis through AMMI model

Tolga *et al.* (2018) emphasized the complexity of chickpea cultivation by demonstrating that genotype x environment interactions significantly influence grain yield and its associated components. Through an evaluation of 18 genotypes, the study found that stability varied greatly across different traits. For instance, while the genotypes Cevdetbey and Seçkin exhibited consistent performance for flowering duration across all tested environments, no high-yielding genotypes maintained high stability for grain yield or pod count. Furthermore, Seçkin demonstrated adaptability regarding plant height and, along with the genotype Er, showed relatively stable performance for 100-grain weight. Conversely, Antem (indicated as DQÕWH]) was identified as a stable performer specifically for the number of grains per plant, illustrating that stability in one trait does not necessarily guarantee consistency in overall yield.

Bhanu *et al.* (2019) utilized GGE biplot analysis to study 35 chickpea genotypes across four different environments. The "Which-won-where" polygon view of the biplot successfully identified the two distinct mega-environments. They studied how GGE biplots are superior to AMMI for identifying environment-specific adapters, allowing breeders to target specific genotypes for terminal drought-prone regions.

Karimizadeh *et al.* (2020) studied 20 Kabuli chickpea genotypes, employed the Average Environment Coordination (AEC) function of the GGE biplot to identify an "ideal genotype" that successfully balances high mean yield with robust stability. The researchers distinguished between statistical methodologies, noting that while the AMMI model excels at partitioning variance, the GGE approach provides a superior visual diagnostic for breeders. This visual clarity is especially advantageous when selecting genotypes capable of maintaining stable and reliable performance across the volatile environmental conditions typical of Mediterranean climates.

Vadez *et al.* (2021) studied the application of stability analysis beyond yield to include nutritional quality, specifically iron (Fe) and zinc (Zn) content. Utilizing AMMI canalysis, the authors found that while grain yield was highly sensitive to environmental changes, mineral density remained relatively stable. Their biplot analysis identified specific biofortified chickpea lines that maintained high mineral levels in both irrigated and rainfed systems, proving their suitability for wide-scale cultivation and food security initiatives.

Jain *et al.* (2022) utilized the biplot vector view to analyze the relationships between different testing sites, mainly focused on heat-tolerant chickpea lines. By interpreting the angles between environment vectors, the researchers identified redundant testing locations where correlations were high (narrow angles). This finding introduced the concept of "Environment Optimization," suggesting that breeders can reduce the logistical costs of multi-location trials by selecting only the most representative and diverse environments.

Tadesse *et al.* (2023) studied 18 Desi chickpea lines, the authors evaluated the effectiveness of both AMMI and GGE models. While both statistical approaches identified the same stable genotypes, the study concluded that the GGE biplot provided a more intuitive and user-friendly visualization of genotype "desirability" scores. These results support the integration of both models in academic research to ensure a comprehensive and visually clear stability profile for new crop varieties.

Singh *et al.* (2024) evaluated 50 advanced chickpea breeding lines, employed the AMMI-1 biplot plotting grain yield against the first interaction principal component (IPC1) to distinguish genotypes that successfully merge stability with high productivity. A critical take away from their research is the pragmatic observation that stability holds minimal value for producers if it is not accompanied by high yield potential. Consequently, the study advocates for the implementation of a Selection Index (SI), a composite metric that integrates AMMI stability parameters with mean performance to ensure that selected varieties are both reliable and high-yielding for commercial farming.

Fikre *et al.* (2025) focused on the drought-prone regions of East Africa, this study utilized GGE biplot analysis to identify "Stability Hotspots." By applying the "Discrimination vs. Representativeness" view, the researchers were able to pinpoint which test locations provided the most useful data. This allowed the breeding program to optimize resource allocation by focusing on environments that most accurately represent the overall Target Population of Environments (TPE).

Parida *et al.* (2026) shifted the focus to mechanical harvesting requirements by analyzing the stability of plant height and pod clearance. Using the AMMI model, the study found that while an "erect habit" is primarily genetically controlled, the height of the first pod is highly sensitive to environmental interactions. The authors emphasized that for mechanical harvesting to be viable, new chickpea varieties must demonstrate high architectural stability across various sowing dates and conditions.

Varshney *et al.* (2026) studied GGE biplots to compare modern molecular breeding with traditional methods through meta-analysis of lines derived from genomic selection. The findings revealed that genomic selection derived lines often possess higher stability than those selected through traditional phenotypic methods. This research serves as a critical bridge between classical biometric analysis and modern genomic tools, proving that molecular breeding can enhance environmental resilience.

12.4 Diversity study through molecular markers

The implementation of DNA markers offers a significantly more robust alternative to traditional morphological markers, primarily because they remain unaffected by environmental fluctuations. By utilizing diverse molecular profiling techniques such as RAPD, SSR, ISSR, and SRAP researchers can gain highly precise insights into genotypic performance and genetic architecture. Notably, co-dominant markers like Simple Sequence

Repeats (SSRs) are particularly advantageous, as they possess the unique ability to distinguish between heterozygotes and homozygotes. This capability allows for a more granular analysis of genotype and allele frequencies, providing breeders with a sophisticated toolkit for tracking inheritance and maintaining genetic diversity.

Samyuktha *et al.* (2018) evaluated 51 chickpea accessions, researchers utilized 30 SSR markers to dissect the crop's genetic architecture. The study uncovered a high degree of allelic richness, identifying a total of 217 alleles with an average of 7.75 per locus. The marker TA64 was identified as the most powerful diagnostic tool with a Polymorphism Information Content (PIC) of 0.85. By applying Bayesian clustering (STRUCTURE), the team successfully partitioned the germplasm into two distinct genetic groups, demonstrating that SSRs possess a much higher resolving power for identifying subpopulations than traditional morphological markers.

Aggarwal *et al.* (2018) assessed the genetic diversity of 66 local and exotic chickpea cultivars using an extensive set of SSR markers, identifying TA22 and TA28 as the most effective for generating polymorphic bands. The researchers reported a similarity index ranging from 0.43 to 0.97, which revealed a dangerously narrow genetic base in modern cultivated varieties compared to their wild relatives. This finding serves as a critical justification for breeders to incorporate wild germplasm into future hybridization programs to restore genetic variability.

Yadav *et al.* (2019) studied on five prominent Indian chickpea varieties this research used 15 SSR markers to develop a UPGMA dendrogram for molecular fingerprinting. Markers such as ICCM0105 and NCPGR223 were highlighted for their efficiency in distinguishing between closely related varieties. Crucially, the study linked specific allelic patterns to drought tolerance, suggesting that molecular diversity analysis is not just a tool for classification but also a reliable predictor of a variety’s physiological resilience in challenging agro-climatic zones.

Bouri *et al.* (2020) studied on Mediterranean chickpea genotypes; this study utilized 20 SSR markers to develop unique "allelic fingerprints" essential for variety identification. By demonstrating that 75% of genomic SSRs specifically markers like TA113 and TA118—are highly polymorphic, the researchers established a scientific basis for using these tools in Intellectual Property Rights (IPR) and plant variety protection.

Solanki *et al.* (2022) evaluated 50 kabuli chickpea lines using 20 SSR primers, identifying TA180 as a highly diverse marker with a Gene Diversity index of 0.49. The study utilized Jaccard's Similarity Coefficient to quantify genetic relationships, allowing breeders to identify highly divergent parental lines. This data is crucial for designing crossing programs specifically aimed at improving seed size and overall yield through heterosis.

Qulmamatova *et al.* (2024) detected 110 total alleles for screening of Kazakh and ICARDA germplasm using 28 SSR markers. The study's most significant contribution was the discovery of novel genetic variation at loci TA14 and TA142, featuring alleles previously undocumented in global databases. This highlights the ongoing importance of SSR markers in uncovering unique genetic resources within under-represented geographical regions.

Murodova *et al.* (2025) studied using Analysis of Molecular Variance (AMOVA) to reveal that 96% of genetic diversity exists within populations. Integrating phenotypic salt-tolerance screening with 37 SSR markers across 50 genotypes. Furthermore, the researchers employed "In Silico" mapping to position markers like TR20 on physical maps, effectively bridging traditional diversity studies with modern structural genomics.

Gifford *et al.* (2026) addresses Fusarium wilt resistance, this global study utilized 69 polymorphic SSRs to identify 191 alleles. Through Linkage Disequilibrium (LD) analysis, the researchers demonstrated that high-density SSR markers can effectively delineate "resistance blocks" within the chickpea genome. This provides a cost-effective alternative to expensive SNP arrays for programs focused specifically on resistance breeding.

Parida *et al.* (2026) studied targeted plant architecture to facilitate mechanical harvesting. By focusing on a specific set of SSR markers flanking the CaPAR1 gene on Chromosome 4, the researchers proved these markers are highly effective at distinguishing between "erect" and "prostrate" growth habits. This provides a rapid, PCR-based selection method for breeders to identify tall, upright plants compatible with combine harvesters.

12.5 Genome wide association studies

Jannatabadi *et al.* (2014) studied the genetic architecture of seed protein content within a diverse collection of Iranian chickpeas. By employing 23 SSR markers distributed across all linkage groups, the researchers identified specific genetic associations that remained consistent across different environmental conditions. Their findings underscored the reliability of SSR markers for identifying and tracking complex nutritional quality traits, which are often heavily influenced by the environment.

Upadhyaya *et al.* (2016) utilized the ICRISAT mini-core collection, this research focused on the genetic basis of biofortification. The study successfully linked SSR markers to seed mineral concentrations, specifically iron (Fe) and zinc (Zn). By pinpointing specific loci on Linkage Groups 1 and 3 (LG1 and LG3) that account for significant phenotypic variation, this work established a vital genetic foundation for breeding nutrient-dense chickpea varieties to combat hidden hunger.

Rehman *et al.* (2021) investigated association mapping to target drought resilience by focusing on root architecture. By combining SSR and SNP markers, the researchers identified that markers such as NCPGR6 and TA176 are closely linked to deep root systems. These genetic insights are critical for developing cultivars capable of terminal drought tolerance, as deeper roots allow the plant to access moisture in receding water tables during the late growth stages.

Qulmamatova *et al.* (2024) analysed kazakh chickpea germplasm, researchers employed SSR genotyping to dissect the components of yield. They identified highly informative markers, including TA22 on LG4 and TA14 on LG6, which are significantly associated with plant height and the number of productive nodes. These markers serve as valuable tools for breeders aiming to develop high-yielding cultivars by selecting for optimized plant architecture and reproductive capacity.

Al-Aboud and Okasha (2025) Addresses the challenge of soil salinity, this study evaluated 120 accessions for salt tolerance. Their Genome-Wide Association Study (GWAS) revealed novel SSR-trait associations specifically linked to biomass production and seedling vigor under saline stress. The authors concluded that SSR markers remain a highly cost-effective and accessible tool, particularly for resource-limited breeding programs seeking to identify and develop stress-resilient genotypes without the high overhead of dense sequencing.

12.6 Identification of Mechanical harvestable genotypes

Hajibarat *et al.* (2015) focused on the genetic mapping of growth habits and plant height using a combination of SSR, SCoT, and CDDP molecular markers. The researchers determined that SSR markers were superior in terms of reproducibility when identifying the genetic loci responsible for "erect" versus "prostrate" growth. This distinction is vital for breeding programs aimed at developing chickpea varieties suitable for modern mechanical harvesting, where an upright architecture is required.

Samyuktha *et al.* (2018) identified several highly informative markers, including TA5, TA14, and TA64 through a comprehensive analysis of molecular diversity and population structure using 30 SSR markers. With a Polymorphism Information Content (PIC) exceeding 0.8, these markers have become industry standards for organizing germplasm into GWAS (Genome-Wide Association Study) panels. Their high level of polymorphism makes them indispensable tools for categorizing architecture-related traits across diverse chickpea populations.

Parida *et al.* (2026) demonstrated the ongoing utility of traditional markers by using a targeted set of SSRs to validate SNP results derived from GWAS. By confirming the genetic control of secondary branching and pod clearance, the study proved that SSRs remain highly relevant for verifying high-density mapping data. This validation step ensures that the genetic markers identified for plant architecture are accurate and reliable for use in specific, high-precision breeding lines.

13. Materials and Methods

The experimental material consisted of 50 genotypes of chickpea. These genotypes were collected from Pulses Research Station, Junagadh Agricultural University, Junagadh.

13.1 Location : Pulses Research Station, Junagadh Agricultural University, Junagadh

13.2 Season and year of experiment : **Year I (2026-27) -***Rabi* Early Sown (ES)
Rabi Normal Sown (NS)
Year II (2027-28) - *Rabi* Early Sown (ES)
Rabi Normal Sown (NS)

13.3 Experimental design : Randomized Complete Block Design (RCBD)

13.4 Number of replications : 3

13.5 Number of genotypes : 50

13.6 Spacing (cm) : 45 x 10 cm

13.7 Plot size : Single row plot of 3 m length

13.8 Observations to be recorded

Within each plot, five plants were randomly selected and tagged for data collection. To minimize border effects, the terminal plants were excluded from this selection. Observations were recorded for these five plants across each line and replication, with the exception of phenological traits such as days to 50% flowering, days to maturity, and reproductive phase duration, which were recorded on a plot basis. The resulting mean values were utilized for subsequent statistical analysis. To ensure consistency, the selected plants were tagged prior to the emergence of the first flower.

13.8.1. Days to 50% flowering

The number of days from the date of sowing to the stage when 50% of the plants in a plot exhibited their first flower will be documented as days to 50% flowering.

13.8.2. Days to maturity

Days to maturity will be recorded as the interval between sowing and the stage when the plants reach physiological maturity, as indicated by the senescence of leaves and the pods turning a straw-yellow color.

13.8.3. Plant height (cm)

Plant height will be measured in centimeters from the ground level to the tip of the main stem at the time of physiological maturity.

13.8.4. Height of the first pod (cm)

The height of the first pod will be estimated in centimeters from the soil surface to the point of insertion of the lowest pod on the main stem.

13.8.5. Number of primary branches per plant

The total number of branches arising directly from the main stem will be counted for five randomly selected plants and then averaged.

13.8.6. Number of secondary branches per plant

The total number of branches arising from the primary branches will be recorded from five randomly selected plants and the mean value will be calculated.

13.8.7. Number of seeds per pod

The total number of seeds will be counted from five randomly selected plants, and the average number of seeds per pod will be determined.

13.8.8. Number of pods per plant

The total number of pods containing seeds will be counted for each of the five randomly selected plants, and the average will be recorded.

13.8.9. 100-Seed weight (g)

A representative sample of 100 seeds will be taken from the net plot yield of each entry and weighed in grams using an electronic precision balance.

13.8.10. Harvest index (%)

The harvest index will be estimated as the ratio of grain yield to the total biological yield and expressed as a percentage using the following formula:

$$\text{Harvest Index (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

13.8.11. Biological yield per plant (g)

After harvesting, the five selected plants (excluding roots) will be sun-dried for five to eight days. Their total weight will then be recorded in grams to determine the average biological yield.

13.8.12. Seed yield per Plot (g)

The total seed yield from each plot in each replication will be recorded in grams after the harvested material is sun-dried for five to eight days and threshed.

13.8.13. Protein content (%)

The protein content of the harvested grains will be estimated using the standardized single-digest method for nitrogen determination. A 0.5 g sample of finely ground seed material will be digested with 14 ml of concentrated sulfuric acid containing 0.5% selenium powder. The digestion will be carried out in a block digester preheated to 370°C for 2.5 hours. After cooling and dilution with distilled water, the nitrogen content in the aliquots will be determined via distillation with sodium hydroxide using an atomic absorption spectrophotometer (Jones

et al., 1991; Sahrawat *et al.*, 2002). The total nitrogen percentage will then be multiplied by a factor of 6.25 to calculate the crude protein content (Jones, 1941).

14. Statistical analysis

The mean values recorded for all genotypes across all studied characters will be subjected to rigorous statistical analysis to evaluate the following parameters:

14.1 Analysis of variance

To assess the significance of differences among the genotypes for all studied traits, the analysis of variance (ANOVA) technique, as suggested by Panse and Sukhatme (1954), will be employed. The statistical model for the Randomized Complete Block Design (RCBD) is defined as follows:

$$Yij = \mu + g_i + r_j + \varepsilon_{ij}$$

Where,

Yij = Phenotypic value of the genotype in the replication.

μ = Overall population mean (General mean).

g_i = Effect of the genotype.

r_j = Effect of the replication.

ε_{ij} = Random error (uncontrolled variation) associated with the genotype in the replication.

14.2 Parameters of variability

(a) Mean

Could be measured as: $\bar{X} = \frac{\sum X_I}{N}$

Where,

Xi = sum of all the observations

N = Total no. of observations

(b) Range

The range is estimated from difference between character's maximum and minimum value.

$$\textbf{Range = Maximum value - Minimum value}$$

(c) Standard error (SE)

The standard error is a statistical measure that quantifies the accuracy with which a sample distribution represents the true population mean, calculated based on the standard deviation and the sample size.

$$\textbf{Standard error} = \frac{SD}{\sqrt{n}}$$

Where,

SD = Standard deviation

$\sqrt{n}$= Total number of observations

(d) Coefficient of variation (CV %)

The coefficient of variation for different characters will be estimated by the formula suggested by Burton and De Vane (1953).

$$\textbf{CV\%} = \frac{SD}{N} \times 100$$

Where,

SD = Standard deviation

N = Mean of character

(e) Phenotypic variance:

Phenotypic variance calculating given formula:

$$V_P = V_G + V_E$$

Where,

V_P = Phenotypic variance

V_G = Genotypic variance

V_E = Environmental variance.

(f) Genotypic variance:

Genotypic variance calculating given formula:

$$\mathbf{V_G = V_P - V_E}$$

Where,

V_G = Genotypic variance

V_P = Phenotypic variance

V_E = Environmental variance.

(g) Genotypic and phenotypic coefficient of variation:

The genotypic and phenotypic coefficient of variation will be calculated according to the formula given by Falconer (1981).

$$\textbf{Genotypic coefficient of variation (GCV)} = \frac{\sigma^2 g}{\bar{X}} \times \mathbf{100}$$

$$\textbf{Phenotypic coefficient of variation (PCV)} = \frac{\sigma^2 P}{\bar{X}} \times \mathbf{100}$$

Categories of range of variation as given by Sivasubramanian and Madhavamenon (1973).

< 10% = Low

10-20% = Moderate

>20% = High

(h) Heritability (broad sense):

Heritability in the broad sense will be calculated as the ratio of genotypic variance to the total phenotypic variance and expressed as a percentage, as proposed by Falconer (1981).

$$\textbf{Heritability } (h^2 \textbf{ (b)}) = \frac{\sigma^2 g}{\sigma^2 p} \times \mathbf{100}$$

The estimated heritability in the broad sense (bs) will be categorized into three groups as suggested by Johnson *et al.* (1955) to determine the effectiveness of selection for various traits:

0-40% = Low

40-60% = Moderate

>60% = High

(i) Expected genetic advance (GA):

The expected genetic advance (GA) for each trait will be estimated by adopting the formula proposed by Johnson *et al.* (1955):

$$\mathbf{GA = k \times h \times \sqrt{\sigma^2 p}}$$

Where,

h = Heritability in broad sense

k = Selection differential, which is equal to 2.06 at 5% intensity of selection. (Lush, 1949)

$\sqrt{\sigma^2 p}$ = Phenotypic standard deviation

(j) Genetic advance as percent of mean (GAM):

The genetic advance as a percentage of the mean (GAM) for each character will be estimated as suggested by Johnson *et al.* (1955). The calculation will follow the formula:

$$\mathbf{GAM} = \frac{GA}{\bar{x}} \times 100$$

Where,

GA = Genetic advance

$\bar{x}$ = General mean

The range of genetic advance could be understood in following manner as reviewed by Johnson *et al.* (1955).

0-10 % = Low

10-20 % = Moderate

>20 % = High

15. CORRELATION COEFFICIENTS

Correlation coefficients will be utilized to quantify the degree and direction of association between two or more variables. In this genetic study, the genotypic correlation coefficient will measure the inherent association between different traits, providing insight

into the genetic linkage or pleiotropic effects. Conversely, the phenotypic correlation coefficient will reflect the combined influence of both genotypic factors and environmental effects on the observed traits.

For the current investigation, both phenotypic and genotypic correlation coefficients will be calculated for all characters following the methodology described by Al-Jibouri *et al.* (1958). Covariance analysis will be employed to process the data, allowing for a comprehensive evaluation of how various yield components and physiological traits interrelate within the chickpea genotypes.

16. PATH COEFFICIENT ANALYSIS

Path coefficient analysis will be employed to further dissect the interrelationships among the studied traits. As a standardized partial regression coefficient, the path coefficient will measure the direct and indirect effects of one variable upon another, allowing for the partitioning of the correlation coefficient into its individual components.

Following the methodology suggested by Dewey and Lu (1959), both phenotypic and genotypic path analyses will be performed. Specifically, the correlation coefficients of the ten independent variables with seed yield will be utilized to estimate their respective direct and indirect contributions to the final yield. This analysis will provide a clearer understanding of the causal relationships between yield components and their relative importance in determining the overall productivity of the chickpea genotypes.

17. Genome wide association studies (GWAS)

17.1 Assessment of Population Structure

To analyze the population structure, a Bayesian clustering approach will be employed using STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) on a dataset consisting of marker alleles from polymorphic markers. The methodology will involve assigning individuals to subpopulations by testing a range of values, with five independent runs per value to ensure consistency (Evanno *et al.*, 2005). Each simulation will be configured with a 100,000 burn-in period followed by 500,000 MCMC (Markov Chain Monte Carlo) iterations (Porras-Hurtado *et al.*, 2013). To refine the accuracy of the ancestry assignments, the final analysis will incorporate an admixture model and correlated allele frequencies. This approach will allow for the detection of mixed genetic origins and subtle subdivisions across the genotypes (Falush *et al.*, 2003).

17.2 Structure harvest

The likelihood of different K values will be calculated to identify the optimal number of subpopulations within the diversity panel. The ideal value of K will be determined from the uppermost hierarchical level of population structure using the ad-hoc statistic, which is based on the second-order rate of change in the log probability of data between successive K values, as proposed by Evanno *et al.* (2005). Additionally, the criteria described by Pritchard *et al.* (2000) will be utilized to define the optimal K. The most reliable value will be estimated based on the lowest negative Ln P(D) (the log-likelihood of the data) and the minimum standard deviation observed during the statistical iterations. This analysis will be performed using the Structure Harvester web-based utility (Earl and VonHoldt, 2012), which processes the output result files generated by STRUCTURE 2.3.4.

17.3 Association Mapping

The association between molecular markers and phenotypic traits will be analyzed using TASSEL version 2.1 software (Bradbury *et al.*, 2007). Two distinct association mapping approaches—the General Linear Model (GLM) and the Mixed Linear Model (MLM)—will be implemented within the TASSEL environment. TASSEL utilizes a fixed-effects linear model to test for associations between marker genotypes and observed phenotypes. To account for population structure, Principal Component Analysis (PCA) will be incorporated into the analysis. For each trait and marker combination, a separate model will be constructed and solved. All auxiliary variables, including factors, covariates, replications, or locations, will be included in the model as main effects. To further refine the association mapping and reduce false positives (type I errors), the MLM approach will be employed. This model incorporates kinship (K) information along with population structure (Q or PCA) to account for relatedness among genotypes. The MLM is defined by the following equation:

$$y = X\beta + Pv + u + e$$

Where,

y = Vector of phenotypic values for the studied traits.

X = Incident matrix of marker genotypes.

β = Vector of fixed effects for the marker genotypes to be estimated.

P = Known matrix of population structure (Q-matrix or PCA components).

v = Vector of fixed effects due to population structure.

u = Vector of random genetic effects (incorporating the kinship matrix to account for relatedness).

e = Vector of random residual errors.

The variances of u and e are given by:

$$Var(u) = 2KVg \text{ and } Var(e) = VR$$

Where,

K = Kinship matrix inferred from genotypes

Vg = Genetic variance

VR= Residual variance.

Markers exhibiting a p-value less than (where represents the total number of markers) will be considered significantly associated with the phenotypic trait. This Bonferroni correction threshold will be applied to control the genome-wide false discovery rate and ensure the reliability of the identified marker-trait associations.

Where,

N= Number of polymorphic markers

17.3.1 QQ-plot development

Quantile-Quantile (Q-Q) plots will be generated using the R package Q-Q plotr (Almeida *et al.*, 2018), utilizing the p-values derived from both the GLM and MLM output files. These plots will be used to visualize the distribution of observed p-values against the expected null distribution to assess the adequacy of the population structure and kinship corrections in the association analysis.

17.3.2 Manhattan plot development

Manhattan plots will be generated using the R package Q-Q man (Turner, 2018), utilizing the p-values and physical marker positions derived from the GLM and MLM output files. These plots will be used to visualize the significance of marker-trait associations across the entire genome, allowing for the identification of potential genomic regions or quantitative trait loci (QTLs) associated with the target traits.

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